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TITLE: Oxytocin and Social Support as Synergistic Inhibitors of Aversive Fear Conditioning and Fear-Potentiated Startle in Male Rats

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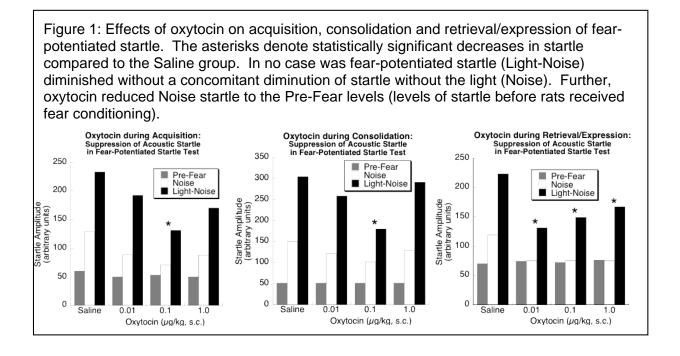
INTRODUCTION

PTSD can be considered a disorder of affective memory where reminiscence of aversive events becomes exaggerated, uncontrollable and frightening. Fear during PTSD also becomes generalized where it is not confined to the trauma, but occurs in other situations or stimuli too. While classic antianxiety and antidepressant drugs have some efficacy for PTSD, newer medications via novel mechanisms are needed. Exogenous oxytocin, a nonapeptide found naturally in the brain and body, may have anti-anxiety effects in animals and humans, and therefore may be effective in interfering with acquisition and retention of aversive memory and promoting extinction. To test this hypothesis in an animal model of PTSD, fear-potentiated startle in male rats was employed. FPS in rats has face-validity for PTSD because a major hallmark symptom of PTSD in humans is exaggerated startle.

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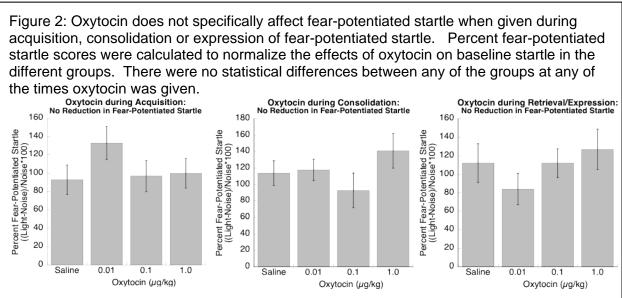
Completed work (Task 1): Oxytocin Reduces Anxiety-Related Increases in Startle, But Not Cue Specific Fear-Potentiated Startle in Rats (manuscript in preparation).

A standard fear-potentiated startle paradigm was used with 12 rats per group in all experiments. In brief, rats were first habituated to acoustic startle stimuli for three days (pre-fear startle). Fear conditioning then occurred with one session of five light-footshock pairings. Four days later, fear-potentiated startle testing occured where acoustic startle stimuli were delivered in the presence or absence of the light. Greater startle in the presence of the light than in its absence was considered cue-specific conditioned fear. Comparisons of pre-fear startle and startle in the absence of the light (Noise) is a measure of background or generalized anxiety. Greater startle in the absence of the light than during the pre-fear startle sessions is considered increased generalized anxiety. A Dunnett's test was used to statistically compare the doses of oxytocin to that of saline. P<0.01 for all analyses.



To test for the effects of oxytocin on fear conditioning and fear-potentiated startle, oxytocin $(0.01, 0.1, 1.0 \,\mu\text{g/kg}, \text{sc})$ was injected 30 minutes before the light-footshock pairings (acquisition), immediately after the light-footshock pairings (consolidation), or 30 minutes before the fear-potentiated startle test (fear retrieval/expression).

These experiments demonstrated that oxytocin given either during acquisition, consolidation, or expression diminished acoustic startle (Figure 1). The effects were not specific to startle in the presence or absence of the conditioned light stimulus. There was no oxytocin effect on fear-potentiated startle (Figure 2). The results could be interpreted as an effect of oxytocin on the ability to startle or perceive the acoustic stimulus. Another interpretation is that oxytocin diminished background or generalized anxiety induced by the fearful situation.



To test these two hypotheses, additional experiments were conducted. In the first experiment, rats were not fear conditioned. Acoustic startle was first tested without oxytocin and then tested again with oxytocin. There was no effect of oxytocin on acoustic startle (Figure 3). This indicates that oxytocin does not diminish the sensory perception or startle response to the acoustic stimulus.

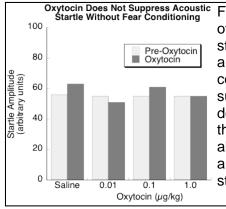
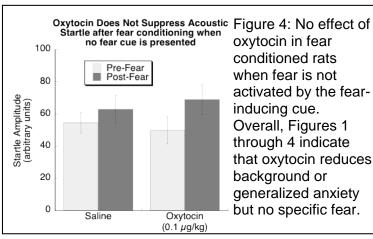


Figure 3: No effect of oxytocin on startle when rats are not fear conditioned. Data suggest oxytocin does not suppress the perception or ability to startle to an acoustic startle stimulus anxious.

In the second experiment, rats were fear conditioned (5 light-footshock pairings), but no light was presented during the acoustic startle testing. There was no effect of oxytocin on acoustic startle when the conditioned fear stimulus was never presented during the testing (Figure 4). Comparing the effect of oxytocin when the situation was made fearful by presenting the light (Figure 1) and when



oxvtocin in fear conditioned rats when fear is not activated by the fearinducing cue. Overall, Figures 1 through 4 indicate that oxytocin reduces background or generalized anxiety but no specific fear.

the light was never presented during the startle session (Figure 4), it is concluded that oxytocin diminishes background or generalized anxiety induced by cue-specific fear (Noise and Light-Noise trials in Figures 1 & 2), but does not reduce the explicit fear to the learned stimulus (percent of fear-potentiated startle in Figure 2).

The experiments suggest that oxytocin has therapeutic

implications for PTSD as a unique anti-anxiety drug that relieves generalized or background anxiety, but leaves specific fear unaffected.

Negative results (Task 1): The effects of social support on the effects of oxytocin on fearpotentiated startle were tested in one experiment. Comparison of rats housed in pairs versus isolated rats did not yield differences on the effects of oxytocin on fear-potentiated startle or background anxiety. This experiment needs to be replicated before conclusions can be drawn.

Uncompleted experiments (Task 1): The effects of oxytocin given either intracerebroventricularly (ICV) or into the amygdala (data not show) have begun and are still underway. So, far there is no effect of ICV administration at several doses tested (Figure 5).

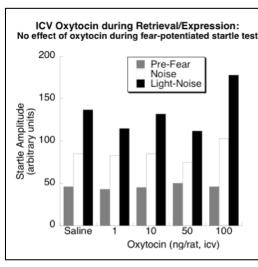


Figure 5: Lack of an effect of several doses of oxytocin given ICV on background or generalized anxiety (Noise) or fearpotentiated startle (Light-Noise).

However, the highest dose tested, 100 ng, suggests that higher doses might increase startle. We still need to test higher doses. Problems with amygdala injections have been found where the saline injections reduce fearpotentiated startle. We will work on overcoming this problem.

Deviations from the original SOW: I do not believe we will be able to complete the in situ

hybridization or receptor binding experiments originally proposed in Task 2. The additional behavioral pharmacology experiments that were necessary following the results from the fear-potentiated startle experiments, additional ICV and intracranial amygdala infusion experiments necessary to identify appropriate doses of oxytocin, and experiments to solve the amygdala infusion problems will likely reduce the funds available for in situ hybridization or

receptor binding experiments. Nevertheless, we will complete corticosterone assays proposed in Task 2. If funds are still available after completion of the behavioral pharmacology studies, as much as possible of the *in situ* hybridization and/or receptor binding experiments will be performed.

Training: A graduate and an undergraduate student were supported by the grant and have worked in collaboration on the completed studies. Each had no experience in behavioral pharmacology or fear-potentiated startle. The undergraduate (Galen Missig) has presented posters of the research in two University of Delaware forums (the university summer undergraduate research forum and the Department of Psychology undergraduate research forum). The graduate student (Luke Ayers) has presented the studies in the department's seminar series and will be presenting the final version of the studies in a poster at the Society for Neuroscience meeting in Chicago in October. Both will be authors on the published manuscript and participate in all aspects of its production including data collection and analysis, and writing the final article.

KEY RESEARCH ACCOMPLISHMENTS

- Systemically administered oxytocin is an effective antianxiety agent in male rats with unique properties of decreasing generalized anxiety but not cue-specific fear.
- Using a fear-potentiated startle paradigm for conditioned fear, oxytocin was effective in reducing acoustic startle.
- Effectiveness of oxytocin was not confined to one phase of fear learning or expression. It reduced startle when given either before fear conditioning (acquisition, immediately after fear conditioning (consolidation) or during fear expression/retrieval (during fear-potentiated startle testing).
- The reduction is startle was general, in that both startle in the presence of the conditioned fear stimulus and in its absence was diminished. However, cue-specific fear was still evidence.
- Further experiments demonstrated that oxytocin did not merely reduce the ability to startle or perceive the acoustic startle stimulus.
- Additional experiments demonstrated that oxytocin only reduced startle in a fear-inducing situation.
- Taken together, it is concluded that oxytocin uniquely inhibits generalized anxiety, while leaving fear to a specific fear stimulus intact.
- The research might have implications for oxytocin as a novel therapeutic treatment for PTSD, which there is a high degree of generalization of fear and anxiety.

KEY TRAINING ACCOMPLSHMENTS

- Graduate and undergraduate students (one each) have been trained in behavioral pharmacology using fear-potentiated startle as a paradigm for testing antianxiety drugs.
- They have learned the proper procedures for conducting animal research.
- They have learned how to analyzes data, construct posters, and write abstracts and manuscripts.
- The students have learned presentation skills, and will present the research at local and national (Society for Neuroscience meeting) research forums.

REPORTABLE OUTCOMES

- 1. Rosen, J.B., Missig, G., and L.W. Ayers (2009). Oxytocin reduces anxiety-related increases in startle, but not cue-specific fear-potentiated startle in rats. Presented at the Military Health Research Forum, Kansas City, MO, August 31-Septmeber 3. Oral presentation # S3-11 and poster presentation # P19-13.
- 2. Ayers, L.W., Missig, G., Schulkin, J., and Rosen, J.B. (2009). Oxytocin reduces anxiety-related increases in startle, but not cue-specific fear-potentiated startle in male rats: Relevance to PTSD. Presented at the Society for Neuroscience meeting, Chicago, IL, October 17-21. Program # 841.17.

CONCLUSION

The project has been quite successful. A manuscript is preparation which will present the unique antianxiety effect of oxytocin discovered in our experiments with fear-potentiated startle in male rats. I believe the report will have a substantial impact, as there is tremendous interest in the role of oxytocin in emotions and mental health. Additional experiments are still ongoing to discover a neural locus of action of oxytocin. Participation and training of graduate and undergraduate students has also been successful. Future plans are to leverage the data and results for submissions of a NIH grant proposal and other grant mechanisms through the Department of Defense.

Appendix 1:

Military Health Research Forum Abstract

OXYTOCIN REDUCES ANXIETY-RELATED INCREASES IN STARTLE, BUT NOT CUE-SPECIFIC FEAR-POTENTIATED STARTLE IN RATS

Jeffrey B. Rosen, Galen Missig, and Luke W. Avers

University of Delaware

Oxytocin increases trustworthiness and well-being, while decreasing anxious feelings in men and women. Oxytocin, therefore, may have therapeutic value for anxiety disorders, like post-traumatic stress disorder (PTSD). To test this hypothesis, the effects of oxytocin were assessed on fear-potentiated startle in male rats. Because PTSD patients have exaggerated startle responses, fear-potentiated startle in rats has face validity as an animal model to examine the effects of oxytocin on fear-exaggerated startle.

Methods: Fear-potentiated startle male Sprague-Dawley rats (225–250 g) from Charles River were housed in pairs. Startle was measured in a startle-sensitive apparatus. There were three phases of the fear-potentiated startle paradigm. Rats were first given a series of acoustic startle stimuli (95, 105, and 115 dB 50 ms white noise) on three consecutive days to determine their baseline startle amplitude. They then received Pavlovian fear conditioning of five pairings of a 3 s light coterminating with a 500 ms, 0.6 mA footshock. Four days later, rats were tested for long-term memory of conditioned fear by delivering startle stimuli either in the presence or absence of the fearconditioned light. Fear-potentiated startle was defined as higher amplitude startle in the presence of the light compared to startle in its absence. Oxytocin (0, 0.01, 0.1, or 1.0 µg, s.c.) was administered 30 minutes before either fear conditioning, immediately after fear conditioning, or before fearpotentiated startle testing to assess its effects on acquisition, consolidation, and expression of conditioned fear, respectively. Startle amplitude without fear conditioning. The effects of oxytocin also were assessed on acoustic startle without fear conditioning. Rats were given a random series of acoustic startle stimuli on three consecutive days to determine their baseline startle amplitude. Four days later rats received 0, 0.01, 0.1, or 1.0 µg, s.c. oxytocin 30 minutes before another series of acoustic startle stimuli. Differences in startle amplitude before oxytocin and during oxytocin were analyzed.

Results: Oxytocin had similar dose-dependent effects on startle during the fear-potentiated startle test when administered at any of the three phases (acquisition, consolidation, or fear expression). There were no specific effects on fear-potentiated startle. However, startle both in the presence and absence of the light was diminished by 0.1 µg of oxytocin, regardless of when oxytocin was administered. This indicated that acoustic startle, but not fear-potentiated startle, was diminished by oxytocin. To examine whether oxytocin interacted with fear conditioning, oxytocin was tested on startle of rats without prior fear conditioning. There was no effect of oxytocin at any of the doses tested.

Conclusions and Impact: Peripheral administration of oxytocin did not diminish cue-specific conditioned fear, but reduced nonspecific anxiety. The findings suggest oxytocin has unique effects of decreasing generalized anxiety without affecting learning and memory of a specific traumatic event. Oxytocin may have anti-anxiety properties that are particularly germane to the generalization of trauma typically seen in PTSD patients.

This work was supported by the U.S. Army Medical Research and Materiel Command under W81XWH-08-1-0182.

Appendix 2:

Society for Neuroscience Abstract # 841.17

Oxytocin reduces anxiety-related increases in startle, but not cue-specific fear-potentiated startle in male rats: Relevance to PTSD.

Luke W. Ayers, Galen Missig, Jay Schulkin and Jeffrey B. Rosen

Oxytocin reportedly decreases anxious feelings in humans and may therefore have therapeutic value for anxiety disorders, like post-traumatic stress disorder (PTSD). Since PTSD patients have exaggerated startle responses, a fear-potentiated startle paradigm in rats may have face validity as an animal model to examine the efficacy of oxytocin in treating these symptoms. Male Sprague-Dawley rats were used in a 3-phase fear-potentiated startle paradigm. Rats were first given a series of acoustic startle stimuli (95, 105 and 115 dB, 50 ms duration) on 3 consecutive days to determine baseline startle amplitude. They then received Pavlovian fear conditioning of five pairings of a 3 s light co-terminating with a 500 ms, 0.6mA footshock. Four days later, rats were tested for conditioned fear by delivering startle stimuli either in the presence or absence of the fear conditioned light. Fear-potentiated startle was defined as higher amplitude startle in the presence of the light compared to startle in its absence. Oxytocin (0, 0.01, 0.1, or 1.0 µg, s.c.) was given 30 min before fear conditioning, immediately after fear conditioning, or 30 min before fear-potentiated startle testing to assess its effects on acquisition, consolidation and expression of conditioned fear, respectively. Startle both in the presence and absence of the light was significantly diminished by oxytocin (0.1 µg/kg) when administered at any of the three phases (acquisition, consolidation, or fear expression). There was no specific effect on fear-potentiated startle. Oxytocin also had no effects on acoustic startle during testing without previous fear conditioning. Further, in a context-conditioned test, previous light-shock fear conditioning did not increase acoustic startle during testing when the light was not presented. The data suggest that oxytocin did not diminish cue-specific conditioned fear, nor contextual fear, but reduced nonspecific anxiety. This suggests that oxytocin has unique effects of decreasing generalized anxiety without affecting learning and memory of a specific traumatic event. Oxytocin may have antianxiety properties that are particularly germane to the generalized hypervigilance and exaggerated startle typically seen in PTSD patients.

Supported by: Department of Defense Post-Traumatic Stress Disorder/Traumatic Brain Injury Research Program of the Office of the Congressionally Directed Medical Research Programs grant W81XWH-08-1-0182